STUDIES ON ELASTOIDIN. III
ROLE OF DISULPHIDE LINKAGE IN THE HYDROTHERMAL SHRINKAGE OF ELASTOIDIN

K. J. KEDLAYA, N. RAMANATHAN & Y. NAYUDAMMA
Central Leather Research Institute, Madras

The prominent role of disulphide linkage in hydrothermal shrinkage of elastoidin is duscussed. The shrinkage temperature of elastoidin is considerably reduced when treated with lime and arsenic sulphide. The lowering of T_s is less when elastoidin is first treated with lime and then with disulphide linkage breaking agent, which is due to 'immunisation'.

Elastoidin belongs to the collagen class of proteins possessing better hydrothermal stability than mammalian collagen, though its hydroxyproline and proline content is low.[1] The better hydrothermal stability of elastoidin was attributed to the small amount of cystine,[2] hydrogen bridge forming potency of large amounts of tyrosine[3] and peroxide linkages involving tyrosine residues.[2]

If the small amount of cystine (0·35%) contributes significantly to the hydrothermal stability of elastoidin, treatment with a suitable disulphide bond breaking agent should reduce its shrinkage temperature. Lime (L) and arsenic sulphide (AS) paste was chosen as disulphide linkage breaking agent because it does not swell collagen or elastoidin owing to the poor osmotic effect of Ca^{+++} or AS^{+++}[4]. Hence there is little possibility of alkali hydrolysis leading to weakening of the structure of elastoidin. This is also proved herein by the shrinkage behaviour of rat tail tendon (RTT) which does not contain the -S-S- linkage.

The shrinkage temperatures of elastoidin and RTT treated with saturated lime were also determined for ascertaining the influence of lime present in excess in the lime and arsenic sulphide paste on their hydrothermal stability.

Experimental

Preparation of elastoidin: Fibres teased out from shark fish fins were treated with trypsin for 48 hours maintaining the pH at 8·2 and temperature at 37°C. The fibres, after washing well with water, were preserved at 4°C in 0·5*M* saline water.

Preparation of RTT: Teased out fibres were washed well, treated with 0·5*M* salt solution and then preserved at 4°C.

Table 1

SHRINKAGE BEHAVIOUR OF ELASTOIDIN AND RTT

S. No.	Sample No.	Treatment	Property determined	Elastoidin	RTT
1	1	Raw	T_s°C	62	55
	2			64	
2	1	Lime water treated	"	62	56
	2			61	
3	1	Lime water treated & then delimed	"	61	54
	2			61	
4	1	L + AS treated	"	42	56
	2			46	
5	1	L + AS treated and delimed	"	41	54
	2			45	
6	2	Raw	LS (%)	72	76
	2	L + AS treated	"	74	72
	2	L + AS treated and delimed	"	75	74
	2	Saturated lime treated		76	75
7	2	Raw	LR (%)	27	10
	2	L + AS treated	"	10	12
	2	L + AS treated and delimed	"	8	8
	2	Saturated lime treated		24	9
	2	Saturated lime treated and delimed		23	10

LS — Linear shrinkage; LR — Linear recovery.

Preparation of lime and arsenic sulphide paste: 500 g. of quick lime (CaO) powder was treated with 50 g. arsenic sulphide; hot water (70°C) was sprinkled over it when, due to the exothermic reaction, calcium sulphydrate was formed along with arsenic hydroxide, calcium hydroxide, calcium sulphide and arsenic sulphide.[4-6]

Shrinkage measurements: Elastoidin and RTT were thoroughly washed and soaked in water for 30 minutes. They were then treated with lime and arsenic sulphide overnight or for 48 hours. Fibres were washed well with distilled water to free them from the adhering chemicals. Finally they were treated with 1% ammonium sulphate solution to remove the possible traces of lime. The shrinkage temperature, and linear shrinkage and recovery properties of raw limed and delimed fibres were determined by the micro shrinkage method.[7]

The T_s of elastoidin and RTT treated only with lime and then with ammonium sulphate was also determined at the limed and delimed stages for the purpose of comparison.

Results and discussion

It is seen from Table 1 that there is no difference in T_s either (i) between fresh and L or L + AS treated or (ii) between L or L + AS treated and corresponding delimed RTT. While elastoidin treated with lime alone behaved in a similar manner, a significant difference in T_s of raw and L + AS treated elastoidin is noticed; T_s of the latter samples is considerably lower than the former by 18-20°C (sample 1). This difference is quite appreciable.

It may also be seen that linear shrinkage and linear recovery of RTT and linear shrinkage of elastoidin are not affected by lime or lime and arsenic sulphide treatment. But linear recovery of lime and arsenic sulphide treated elastoidin is considerably reduced.

Absence of any difference in T_s of fresh limed or lime and arsenic sulphide treated and corresponding delimed RTT is due to the deliming effect of distilled water on fibres limed by either of the methods.[2] The deliming effect of distilled water is also responsible for the absence of a difference in T_s of raw, lime treated and subsequently delimed elastoidin.

On treating elastoidin with lime and arsenic sulphide paste, calcium sulphydrate [$Ca(SH)_2$] present perhaps reacts with the -S-S- linkage of elastoidin as follows resulting in the rupture of disulpihde bridge lowering the T_s.

$$2\ Ca(SH)_2 + R\text{-}S\text{-}S\text{-}R \longrightarrow 2\ CaS + 2\ R.SH$$

where R represents peptide chains cross bonded together by -S-S- linkage of cystine. [A two step reaction with the intermediate formation of a sulphonic acid is also possible though the net reaction is the same as above.[5]]

Since hydrophobic bond characteristic of the -S-S- bridge of cystine is stable up to about 60°C,[7] rupture of disulphide bridge results in disappearance of this characteristic; this phenomenon perhaps aids in lowering the T_s of lime and arsenic sulphide treated elastoidin to a small extent.

Considerable lowering in the linear recovery value of elastoidin on lime and arsenic sulphide treatment indicates the significant role of -S-S- linkage in hydrothermal recovery. This supports the earlier view[8] that the good recovery of denatured elastoidin can be attributed to -S-S- linkage involving cystine. The fact is further confirmed by the less lowering of the T_s (8°C) of elastoidin pretreated with saturated lime water overnight and then retreated with lime and arsenic sulphide for 24 hours. The fibre was washed, as before, to free it from adhering chemicals before carrying out the T_s measurements.

Pretreatment with $Ca(OH)_2$ perhaps stabilises the -S-S- linkage due to immunisation caused by the formation of $-CH_2$-S-Ca-S-CH_2- bridge containing nonionisable calcium[9] or lanthionine bridge $-CH_2$-S-CH_2-.[10, 11] Obviously T_s of RTT subjected to similar treatment is not affected due to the absence of the -S-S- linkage. Hence it is concluded that the disulphide linkage in elastoidin plays

a prominent role in its hydrothermal stability.

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